



Attorney Docket Number 54763-8001US02

## EXHIBIT B

PAGES FROM

LABORATORY NOTEBOOK

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Drug Sensitivity and growth curves

Cisplatin test.

6 wells per line x 2 → 12 wells for duplicates.

Concentrations:

- 0
- 0.1  $\mu M$
- 0.5 "
- 2.0 "
- 10.0 "
- 50.0 "

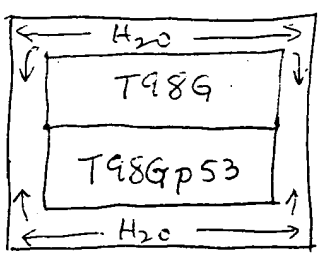


plate  $2 \times 10^4$  cells per well, settle overnight.

treat 1 hour w/ cisplatin = 1 hr. 15 min.

Count cells on

Growth curve

6 wells per line x 2 → 12 wells.

2000 cells per well.

T98Gp53 (culture previously assayed on ) kept without G418 since

1 almost confluent 6 cm dish trypsinized and resuspended in 5 ml.

$$\frac{103 \text{ cells}}{9 \text{ squares}} = 11 \times 10^4 \text{ per ml.}$$

$$\begin{aligned} 11,000 / 100 \mu l. \\ 22,000 / 200 \mu l. \leftarrow \text{per well. 48 well dish.} \end{aligned}$$

for drug test, add 200  $\mu l$  per well + 500  $\mu l$  medium.

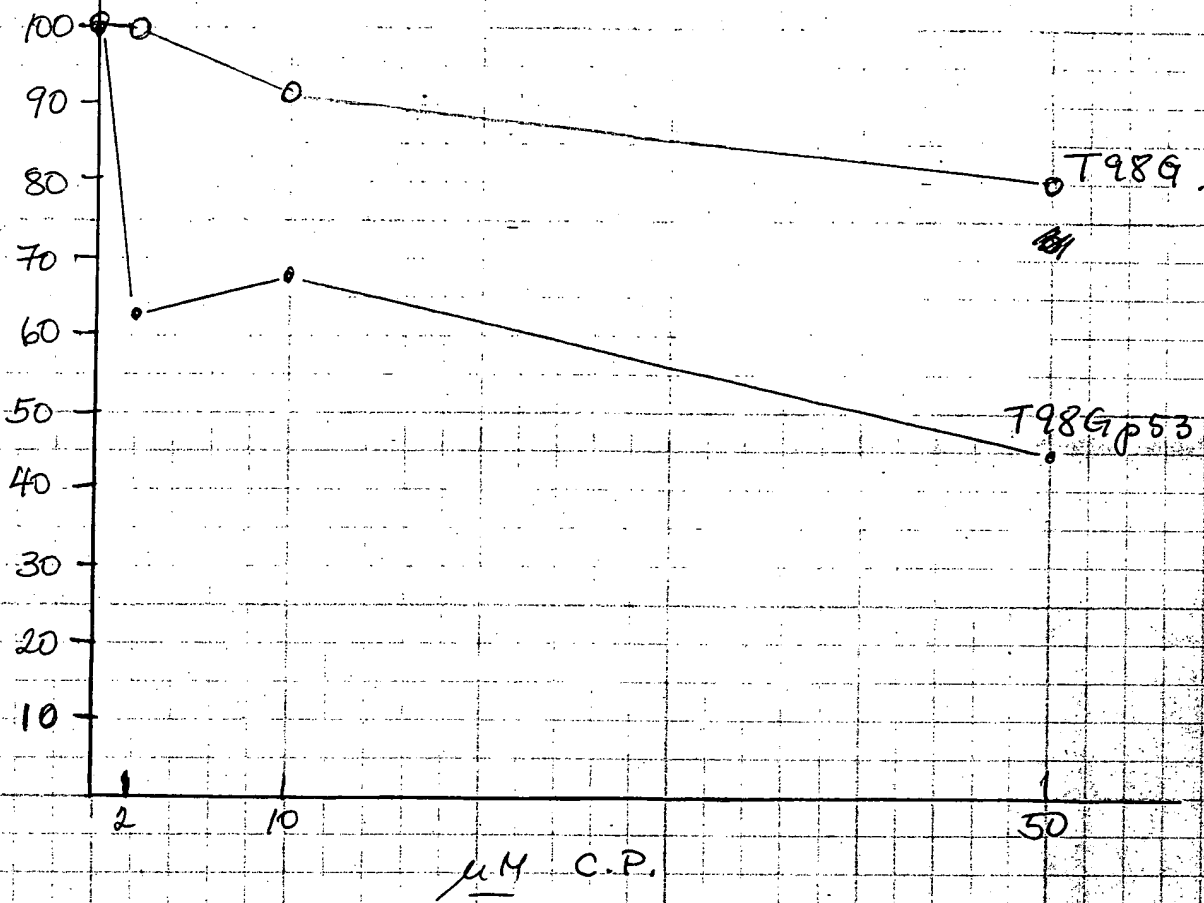
for growth curve, plate 1500 cells per well.

$$\begin{aligned} 11,000 \text{ cells} / 100 \mu l. \\ 1500 \text{ cells} / 14 \mu l. \end{aligned}$$

196  $\mu l$  (14 wells) into 6.8 ml. → 7 ml total, 0.5 ml per well.



# Cisplatin sensitivity

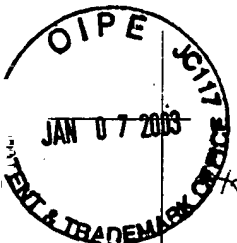


cells plated in 48 well plates 2 wells per point, at 20,000 cells per well (very dense).

cells treated 1 hr w/ CP medium changed, and cell counts were made one week later.

plating  
treat  
counts.

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Cisplatin test. T98G, T98G p53. (Growth w/o 6418)

Stock Cisplatin in DMEM at 4 mM.

treatment done in 48-well plates, 0.5 ml per well.  
170,000 cells per well plated out on previous day

prepare 2 mls each concentration:

10 $\mu$ M	add 5 $\mu$ l stock (5 $\mu$ l $\cdot$ 4 mM in 2 ml.)
20 $\mu$ M	10 $\mu$ l "
30	" 15 $\mu$ l.
40	20
60	30.
120	60
240	120.

following 1 hr. treatment, trypsin cells w/ 100  $\mu$ l trypsin, add 40  $\mu$ l complete medium; pipet up and down w/ 100  $\mu$ l tip to disperse, then replat in duplicate

5  $\mu$ l  $\rightarrow$  2 plates ( $\sim$  100 colonies)  
and/or 100  $\mu$ l  $\rightarrow$  2 plates ( $\sim$  2000 colonies).

as follows:

control	(0)	100 (x2 plates)	2000 (x2 plates)
10 $\mu$ M		"	"
20 $\mu$ M		"	"
30 $\mu$ M		"	"
40 $\mu$ M		—	"
60 $\mu$ M		—	"
120 $\mu$ M		—	"
240 $\mu$ M		—	"

8 plates

16 plates.

24 plates  
per line.

The remaining cells in 48 well dish were allowed to continue to grow and were counted on

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1484 p<sup>3</sup>

10  $\mu$ M 68 x 4 x 10<sup>4</sup> / ml.

60  
48  
66  
(122)  
242

20  $\mu$ M 53  
56 (120)  
64

30  $\mu$ M 24  
22 (96)  
26

40  $\mu$ M  
( $\frac{16}{3}$ ) x 4 x 10<sup>4</sup>  
( $\frac{17}{3}$ ) (25)  
( $\frac{23}{3}$ )

60  $\mu$ M  $\frac{3}{9}$  x 4 x 10<sup>4</sup> / ml. (1.3)

120  $\mu$ M ( $\frac{1}{9}$ ) (0.4)

240  $\mu$ M  $\frac{1}{9}$  (0.4)

0 41

% max # cells.

10  $\mu$ M 100%  
20  $\mu$ M 95%  
30  $\mu$ M 40%  
40  $\mu$ M 10%  
60  $\mu$ M 0.5%  
120  $\mu$ M 0.17%  
240  $\mu$ M "

CELL CENTER 1005250

T 989C = 2

~~88~~ x 4 x 10<sup>4</sup> / ml.

40  $\mu$ g

85  
92 (364) 88%

~~83~~  
97  
110  
104  
(383)  
(415) 100%

60  $\mu$ g

23

10  $\mu$ g  
115  
110  
97  
(429) 103

19  
17  
25  
84 20%

20  $\mu$ g  
85  
~~77~~

104  
106  
84  
(365) 88  
379 91

120  $\mu$ g  
23  
9 10 2.4%

240  $\mu$ g  
4  
9 1.8 0.4%

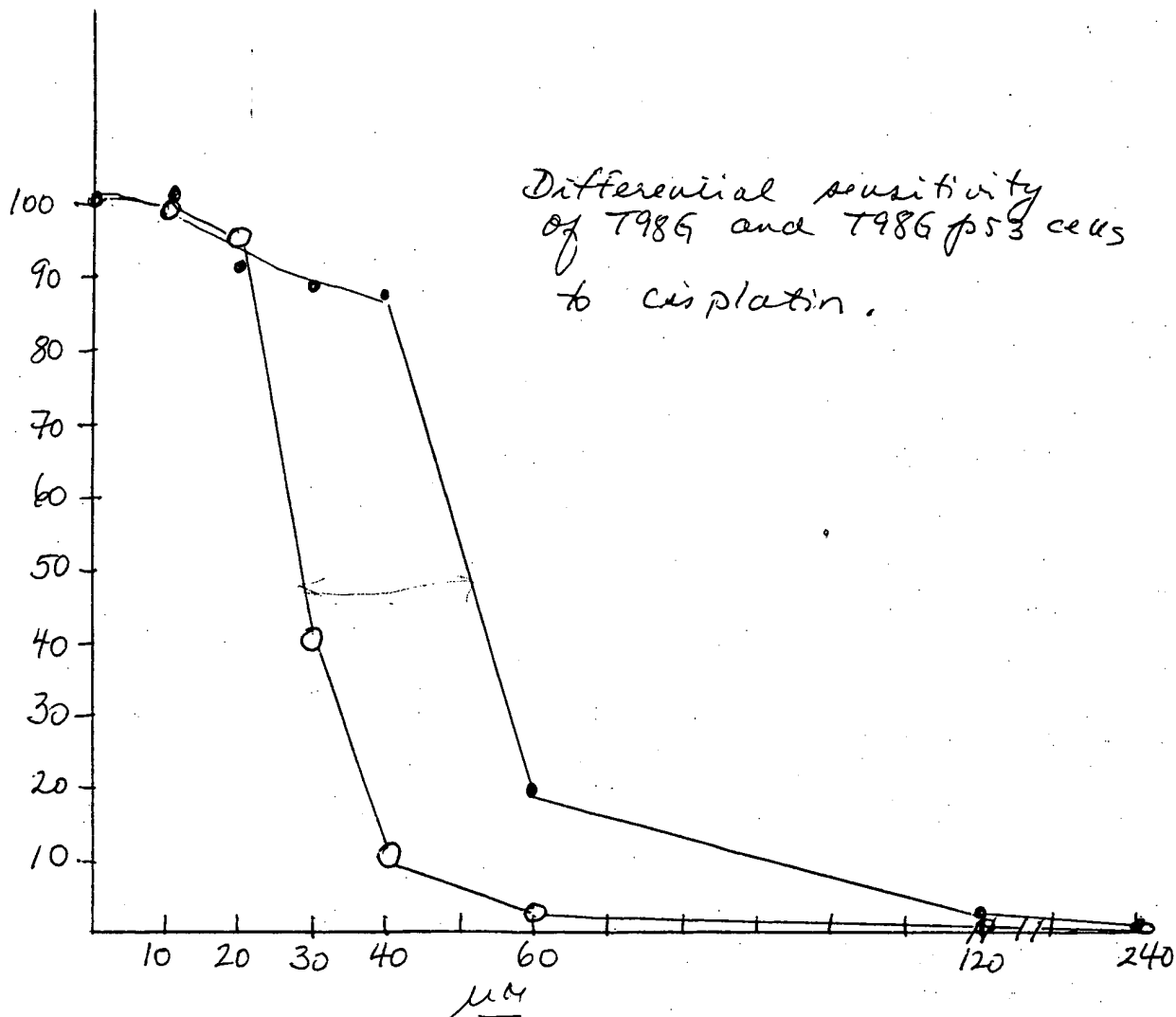
30  $\mu$ g  
95  
85  
(407) 98  
89

~~77~~  
97

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FEDERAL BUREAU OF INVESTIGATION



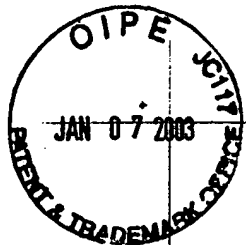
42-381 50 SHEETS 5 SQUARE  
42-382 100 SHEETS 3 SQUARE  
42-389 200 SHEETS 5 SQUARE  
MINI-USA



E.C.50  $\sim 27 \mu\text{M}$  T98G p53

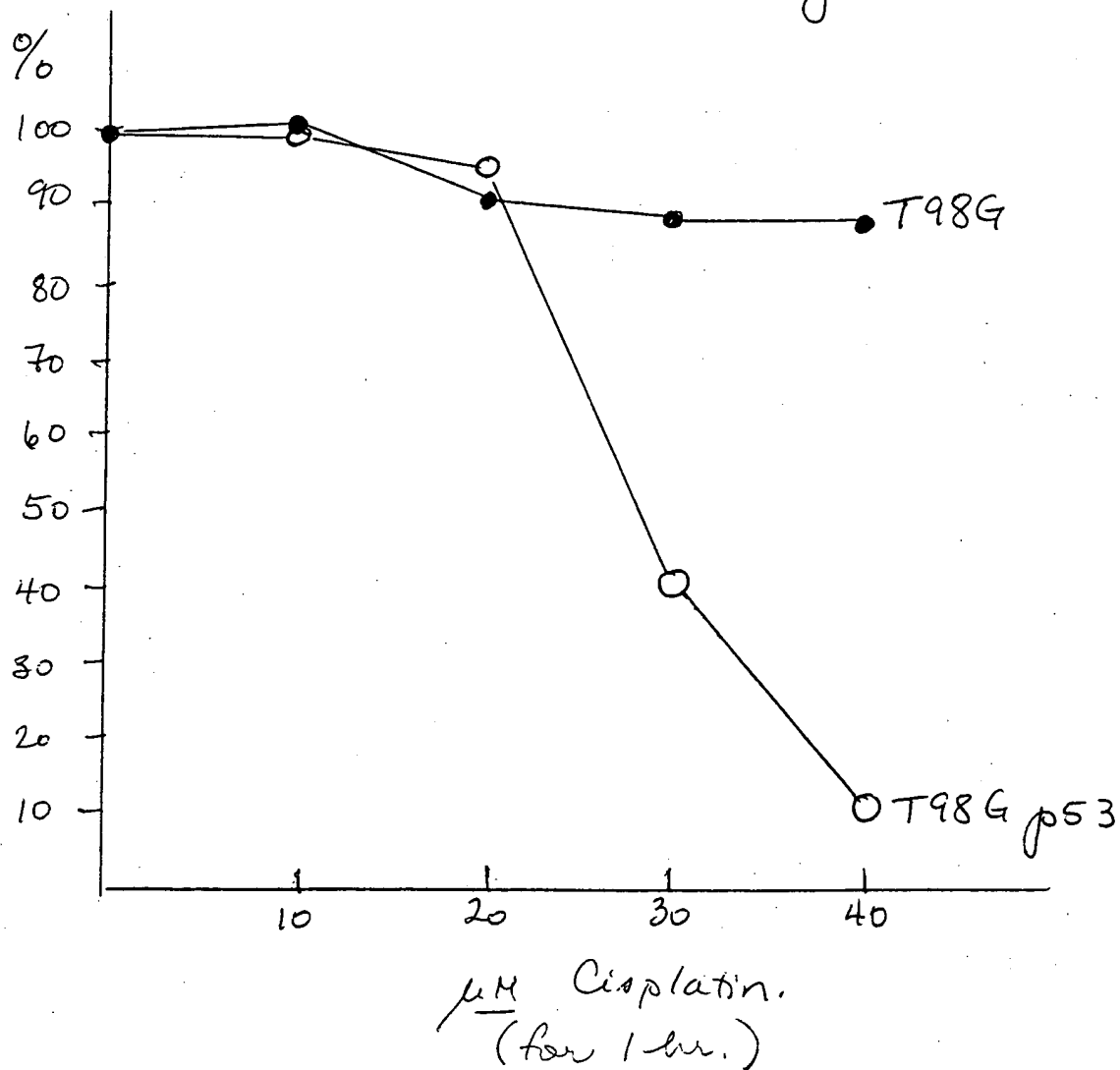
EC 50  $\sim 50 \mu\text{M}$  T98G.

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FEB 11 1984  
TECHNICAL SERVICES  
Cells treated 1 hr. with varying doses of cisplatin,  
trypsinized and settled in same wells; counted  
one week later.



# Cisplatin sensitivity of T98G cells. T98Gp53

% maximum cell growth at 7 days



$\mu\text{M}$  Cisplatin.  
(for 1 hr.)

42.381 50 SHEETS 5 SQUARE  
42.382 100 SHEETS 5 SQUARE  
42.389 200 SHEETS 5 SQUARE



NATIONAL

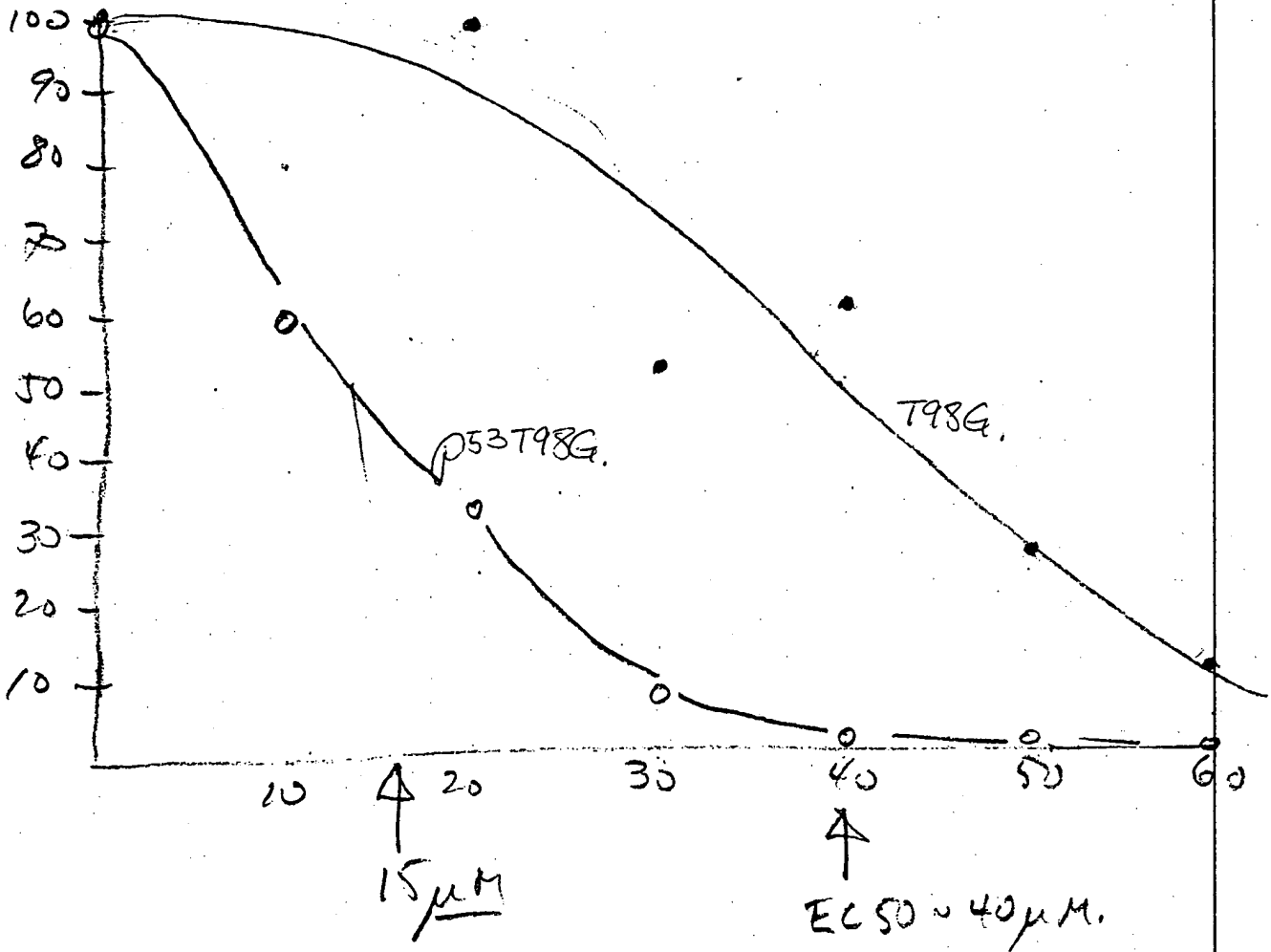
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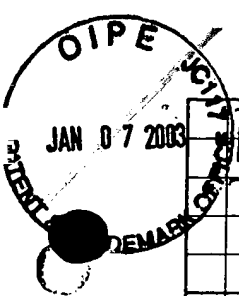




Taxon's Repeat of the Cisplatin  
Sensitivity experiment.



42-381 50 SHEETS EYE-EASE® 5 SQUARE  
42-382 100 SHEETS EYE-EASE® 5 SQUARE  
42-383 200 SHEETS EYE-EASE® 5 SQUARE  
42-384 50 SHEETS EYE-EASE® 10 SQUARE  
42-385 100 SHEETS EYE-EASE® 10 SQUARE  
42-386 200 SHEETS EYE-EASE® 10 SQUARE  
42-387 50 SHEETS EYE-EASE® 20 SQUARE  
42-388 100 SHEETS EYE-EASE® 20 SQUARE  
42-389 200 SHEETS EYE-EASE® 20 SQUARE  
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42-397 100 SHEETS EYE-EASE® 160 SQUARE  
42-398 200 SHEETS EYE-EASE® 160 SQUARE  
42-399 50 SHEETS EYE-EASE® 320 SQUARE  
42-400 100 SHEETS EYE-EASE® 320 SQUARE  
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42-402 50 SHEETS EYE-EASE® 640 SQUARE  
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42-621 50 SHEETS EYE-EASE® 6044629098073145873530880 SQUARE  
42-622 100 SHEETS EYE-EASE® 6044629098073145873530880 SQUARE  
42-623 200 SHEETS EYE-EASE® 6044629098073145873530880 SQUARE  
42-624 50 SHEETS EYE-EASE® 12089258196146291747061760 SQUARE  
42-625 100 SHEETS EYE-EASE® 12089258196146291747061760 SQUARE  
42-626 200 SHEETS EYE-EASE® 12089258196146291747061760 SQUARE  
42-627 50 SHEETS EYE-EASE® 24178516392292583494123520 SQUARE  
42-628 100 SHEETS EYE-EASE® 24178516392292583494123520 SQUARE  
42-629 200 SHEETS EYE-EASE® 24178516392292583494123520 SQUARE  
42-630 50 SHEETS EYE-EASE® 48357032784585166988247040 SQUARE  
42-631 100 SHEETS EYE-EASE® 48357032784585166988247040 SQUARE  
42-632 200 SHEETS EYE-EASE® 48357032784585166988247040 SQUARE  
42-633 50 SHEETS EYE-EASE® 96714065569170333976494080 SQUARE  
42-634 100 SHEETS EYE-EASE® 96714065569170333976494080 SQUARE  
42-635 200 SHEETS EYE-EASE® 96714065569170333976494080 SQUARE  
42-636 50 SHEETS EYE-EASE® 193428131138340667952988160 SQUARE  
42-637 100 SHEETS EYE-EASE® 193428131138340667952988160 SQUARE  
42-638 200 SHEETS EYE-EASE® 193428131138340667952988160 SQUARE  
42-639 50 SHEETS EYE-EASE® 386856262276681335905976320 SQUARE  
42-640 100 SHEETS EYE-EASE® 386856262276681335905976320 SQUARE  
42-641 200 SHEETS EYE-EASE® 386856262276681335905976320 SQUARE  
42-642 50 SHEETS EYE-EASE® 773712524553362671811952640 SQUARE  
42-643 100 SHEETS EYE-EASE® 773712524553362671811952640 SQUARE  
42-644 200 SHEETS EYE-EASE® 773712524553362671811952640 SQUARE  
42-645 50 SHEETS EYE-EASE® 1547425049106725343623905280 SQUARE  
42-646 100 SHEETS EYE-EASE® 1547425049106725343623905280 SQUARE  
42-647 200 SHEETS EYE-EASE® 1547425049106725343623905280 SQUARE  
42-648 50 SHEETS EYE-EASE® 3094850098213450687247810560 SQUARE  
42-649 100 SHEETS EYE-EASE® 3094850098213450687247810560 SQUARE  
42-650 200 SHEETS EYE-EASE® 3094850098213450687247810560 SQUARE  
42-651 50 SHEETS EYE-EASE® 6189700196426901374495621120 SQUARE  
42-652 100 SHEETS EYE-EASE® 6189700196426901374495621120 SQUARE  
42-653 200 SHEETS EYE-EASE® 6189700196426901374495621120 SQUARE  
42-654 50 SHEETS EYE-EASE® 12379400392853802748991242240 SQUARE  
42-655 100 SHEETS EYE-EASE® 12379400392853802748991242240 SQUARE  
42-656 200 SHEETS EYE-EASE® 12379400392853802748991242240 SQUARE  
42-657 50 SHEETS EYE-EASE® 24758800785707605497982484480 SQUARE  
42-658 100 SHEETS EYE-EASE® 24758800785707605497982484480 SQUARE  
42-659 200 SHEETS EYE-EASE® 24758800785707605497982484480 SQUARE  
42-660 50 SHEETS EYE-EASE® 49517601571415210995964968960 SQUARE  
42-661 100 SHEETS EYE-EASE® 49517601571415210995964968960 SQUARE  
42-662 200 SHEETS EYE-EASE® 49517601571415210995964968960 SQUARE  
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42-664 100 SHEETS EYE-EASE® 99035203142830421991929937920 SQUARE  
42-665 200 SHEETS EYE-EASE® 99035203142830421991929937920 SQUARE  
42-666 50 SHEETS EYE-EASE® 198070406285660843983859875840 SQUARE  
42-667 100 SHEETS EYE-EASE® 198070406285660843983859875840 SQUARE  
42-668 200 SHEETS EYE-EASE® 198070406285660843983859875840 SQUARE  
42-669 50 SHEETS EYE-EASE® 396140812571321687967719751680 SQUARE  
42-670 100 SHEETS EYE-EASE® 396140812571321687967719751680 SQUARE  
42-671 200 SHEETS EYE-EASE® 396140812571321687967719751680 SQUARE  
42-672 50 SHEETS EYE-EASE® 792281625142643375935439503360 SQUARE  
42-673 100 SHEETS EYE-EASE® 792281625142643375935439503360 SQUARE  
42-674 200 SHEETS EYE-EASE® 792281625142643375935439503360 SQUARE  
42-675 50 SHEETS EYE-EASE® 1584563250285286751870879006720 SQUARE  
42-676 100 SHEETS EYE-EASE® 1584563250285286751870879006720 SQUARE  
42-677 200 SHEETS EYE-EASE® 1584563250285286751870879006720 SQUARE  
42-678 50 SHEETS EYE-EASE® 3169126500570573503741758013440 SQUARE  
42-679 100 SHEETS EYE-EASE® 3169126500570573503741758013440 SQUARE  
42-680 200 SHEETS EYE-EASE® 3169126500570573503741758013440 SQUARE  
42-681 50 SHEETS EYE-EASE® 6338253001141147007483516026880 SQUARE  
42-682 100 SHEETS EYE-EASE® 6338253001141147007483516026880 SQUARE  
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42-685 100 SHEETS EYE-EASE® 12676506002282294014967032053760 SQUARE  
42-686 200 SHEETS EYE-EASE® 12676506002282294014967032053760 SQUARE  
42-687 50 SHEETS EYE-EASE® 25353012004564588029934064107520 SQUARE  
42-688 100 SHEETS EYE-EASE® 25353012004564588029934064107520 SQUARE  
42-689 200 SHEETS EYE-EASE® 25353012004564588029934064107520 SQUARE  
42-690 50 SHEETS EYE-EASE® 50706024009129176059868128215040 SQUARE  
42-691 100 SHEETS EYE-EASE® 5070602400912



1946

1986

0 A

$\frac{107}{9}$  }  $12.3 \times 10^4$   
 $\frac{115}{9}$  }

11.5  
100%

$\frac{50}{95}$  } 53.5  
 $\frac{44}{45}$  }

54

0 B

$\frac{93}{9}$  }  $10.7 \times 10^4$   
 $\frac{99}{9}$  }

$\frac{52}{46}$  } 54  
 $\frac{46}{46}$  }

49.3

10 C

$\frac{63}{9}$  } 6.7 58%  
 $\frac{57}{9}$  }

$\frac{379}{9}$  } 42

78%  
85%

20 D

$\frac{42}{9}$  } 4.3 37  
 $\frac{36}{9}$  }

$\frac{55}{63}$  } 60  
 $\frac{46}{56}$  }

55  
56 51.3 100%  
43 110  
49

30 E

$\frac{9}{9}$  } 1.0 9  
 $\frac{46}{9}$  }

$\frac{26}{34}$  } 29.2  
 $\frac{32}{32}$  }

32 54%  
23.2 5

40 F

$\frac{1}{9}$  } 0.11 1  
 $\frac{1}{9}$  }

$\frac{36}{31}$  } 34  
 $\frac{35}{35}$  }

33

61%

50 G

$\frac{1}{9}$  0.4 1

$\frac{18}{20}$  17.5  
 $\frac{20}{20}$  18  
 $\frac{12}{12}$

18

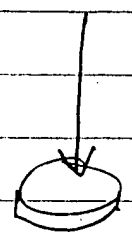
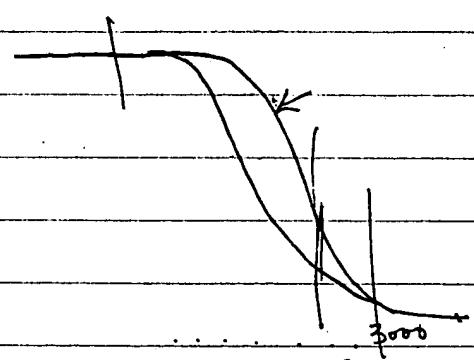
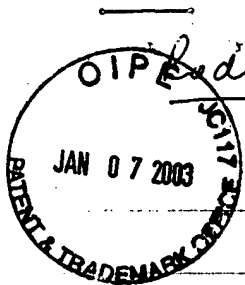
83%  
35

$\frac{1}{9}$  0.05 0.5%

$\frac{4}{16}$  13.5  
 $\frac{14}{14}$  14  
 $\frac{10}{10}$

14

26%  
27



50"  
1000 rads ~~trans.~~  
500 rads 25"  
60 rads ~~3~~"  
3"

$2.3 \times 10^5$  colonies  
10<sup>6</sup> cells  
5.10<sup>5</sup> / T25  
5 x 10<sup>4</sup> / T25

1000  
5 x 10<sup>5</sup>  
20 3000

$\frac{20}{4 \cdot 10^4} \cdot 0.5 \cdot 10^{-3}$

1500 ~ 0.3 x 10<sup>-4</sup>  
 $\frac{1500}{4 \cdot 10^4}$

$\frac{15 \cdot 10^2}{40 \cdot 10^3} \approx 0.3 \cdot 10^{-1}$

T25 25  
50,000 T25  
10,000

2000  
T98G

12 flasks each line.

5000 cells per T25

60	<del>60</del>	3"
80	100	5"
100	200	10"
140	300	15"
180	400	20"
220	500	25"
260	600	30"
300	700	35"
340	800	40"
	900	45"
	1000	50"
	1500	75"

3 series of 12 T25 flasks were set up on

- ① T98G parental line
- ② T98G p53 propagated in G418 until time of plating
- ③ T98G p53 propagated two weeks w/o G418.

Cells were allowed to attach for 24 hrs prior to irradiation.

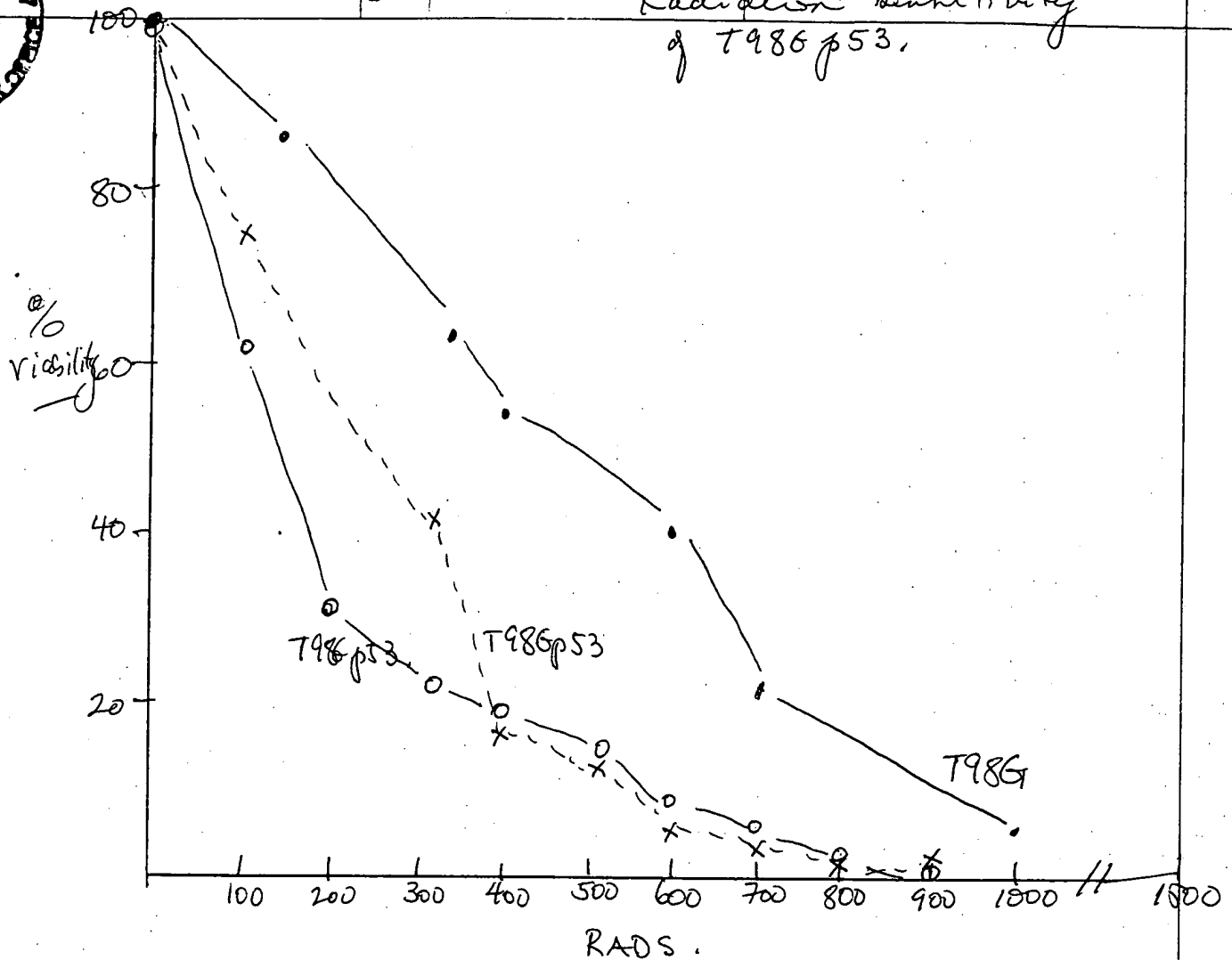


	1	2	3	4	5	6	7	8	9	
1	@ 20cm = 1460 x 1.31 = 1912.6									
2										
3	<u>Dose</u>	<u>Time</u>	<u>Distance</u>	<u>Dose (cgy)</u>						
4	100	3"	25	60						
5	200	6"	25	120*						
6	300	9"	25	179						
7	400	12"	25	239						
8	500	15"	25	299						
9	600	18"	25	359						
10	700	22"	25	438						
11	800	25"	25	536						
12	900	28"	25	695						
13	1000	31"	21	889						
14	1500	47"	21	1348						
15	Control	0	0	0						
16										
17	25cm = 934 x 1.28 = 1195.5									
18	24cm = 1000 x 1.286 = 1286.0									
19	22.5cm = 1150 x 1.294 = 1488.1									
20	21cm = 1320 x 1.307 = 1721.3									
21										
22	* May have receive slightly greater exposure because of technical problems.									
23										
24										
25										
26										
27										
28										
29										
30										

10/10/03  
 10/10/03  
 10/10/03



Radiation Sensitivity  
of T986p53.



Colonies of T986 counted at 5 days.

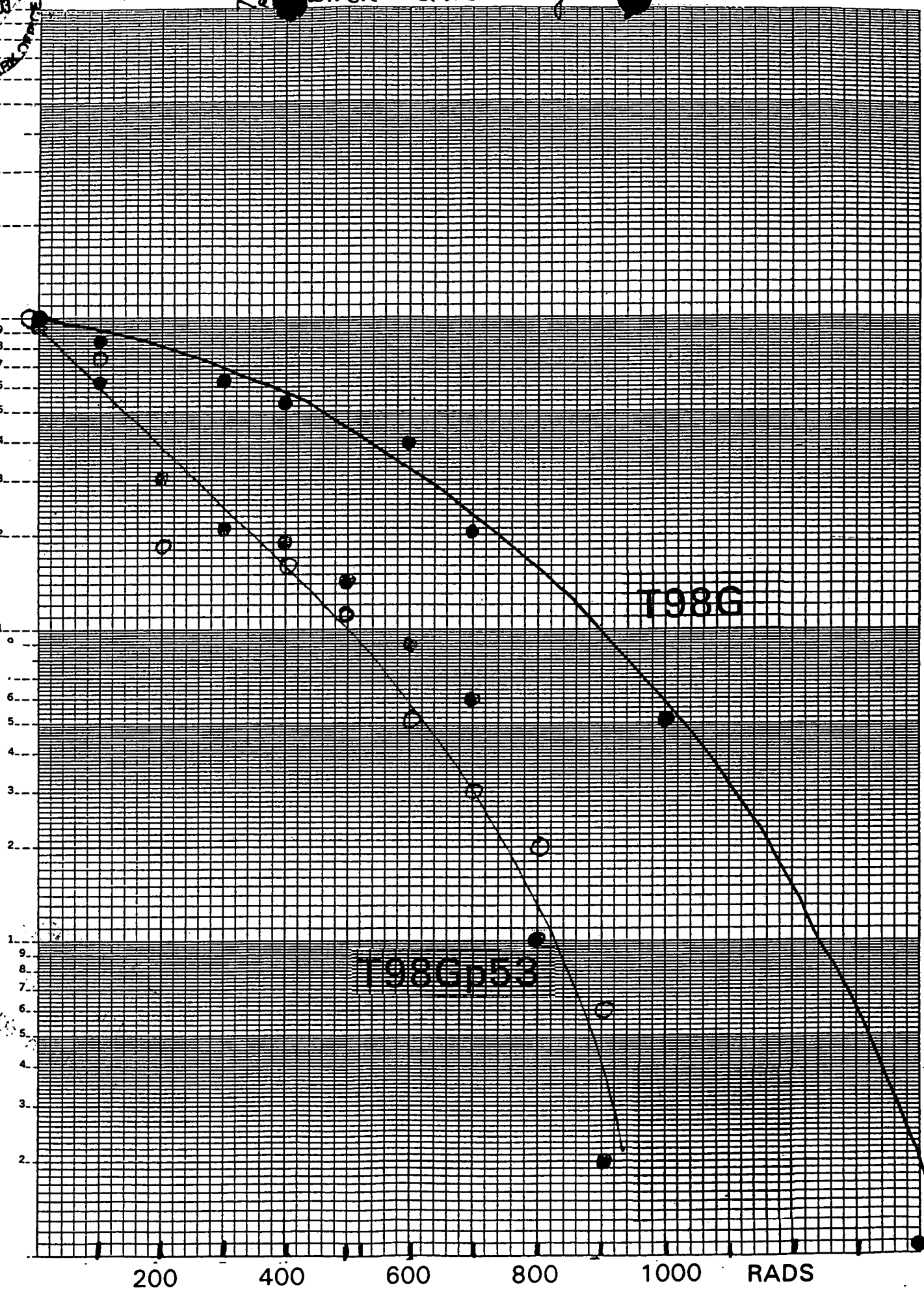
Colonies of T986p53 counted at 12 days.

RECEIVED  
JAN 07 2003  
TECH CENTER  
BENTON & BOWLES

JAN 07 2003  
PATENT & TRADEMARK OFFICE

# Radiation Sensitivity

PERCENT SURVIVAL



T98G

T98Gp53

200 400 600 800 1000 RADS